

**REMARKS**

In the present Amendment, claim 1 has been amended to incorporate the subject matter of claims 3 and 5 and to delete the recitation "without a protease enzyme." Claims 3 and 5 have been cancelled. Claims 4 and 6 have been amended consistent with the amendment to claim 1. Claims 9 and 12 have been amended in a similar manner to claim 1. Claims 15 and 16 have been cancelled in view of the amendment to claim 1. No new matter has been added, and entry of the Amendment is respectfully requested.

Upon entry of the Amendment, claims 1, 4, 6-14 and 20 will be pending.

In paragraph No. 2 of the Action, claims 1, 3-16 and 20 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The Examiner contends that the limitation "without a protease enzyme" is new matter, because there is no express support for this limitation.

As noted, the limitation "without a protease enzyme" has been deleted. Accordingly, withdrawal of the § 112 rejection is requested.

In paragraph No. 5 of the Action, claims 1, 3-16 and 20 are rejected under 35 U.S.C. § 102(b or e) as allegedly being anticipated by or, in the alternative, under 35 U.S.C. § 103(a) as allegedly being unpatentable over Kawamura et al (US 6,344,499) or Galimberti et al (US 2003/0109625).

In paragraph No. 6 of the Action, claims 1, 3-10, 12-16 and 20 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Ichikawa et al (US 2004/0014876).

The above two rejections should be withdrawn because Kawamura et al, Galimberti et al and Ichikawa et al do not disclose or render obvious the present invention.

The cited references disclose decomposing protein in natural rubber latex by using protease and the protease may be combined with other enzymes such as amylase and cellulose.

In the Amendment filed December 27, 2007, Applicant explained that  $\alpha$ -amylase and cellulase exert an enzyme action on the glucans. In contrast, a protease exerts an enzyme action on the protein, and does not exert an enzyme action on the glucans. Applicant further explained that the unexpectedly superior effects provided by the present invention cannot be attained in the combination of protease with enzymes such as amylase and cellulose. Protease exerts an enzyme action on the protein, and therefore,  $\alpha$ -amylase and cellulase themselves are decomposed and lose an enzyme action on the glucans. Therefore, the cited references do not teach or suggest the decomposition of glucans.

In response, the Examiner contends that the art discloses the claimed enzymes and these enzymes can decompose glucans as claimed. Per the Examiner, Applicants have to show or allege that such is not the case.

Applicant responds as follows.

Ichikawa et al discloses that an alkaline protease is mixed with ammonium polyacrylate to obtain a deproteinizing agent in paragraph [0128]. The alkaline protease is especially effective at a pH of 9-10 (see the attached paper 1). Kawamura et al also adapted a pH of 9 as disclosed at line 3 of column 7.

In contrast, Cellulase A “Amano” 3 employed in Example 3 of the present specification has an optimum pH of 4.5 (see the attached paper 2). Biozyme A employed in Example 1 of the present specification has an optimum pH of 5.0. When an amylase and a cellulase are adapted to the pH of 9, the Relative Activity is 10% or less.

Therefore, if the treatment by an alkaline protease is applied to the decomposition of the protein with an amylase and a cellulase, the treatment by an amylase and a cellulase can not be carried out for the decomposition of the glucans because the Residual Activity of the amylase and the cellulase is 10 % or less at a pH of 9.

Accordingly, even if the cited references disclose an amylase or cellulase, the effect for the decomposition of the glucans cannot arise because the Residual Activity of the amylase and the cellulase is very low at the optimal pH value for the decomposition of the protein by protease.

When the glucans contained in the latex are decomposed with  $\alpha$ -amylase, the rubber is excellent in low hysteresis loss property without reducing abrasion resistance and is low in compound Mooney viscosity. Therefore, the rubber is excellent in processability (as shown in Examples 1 and 2, lines 1-7 at page 20 of the specification).

When the glucans contained in the latex are decomposed with cellulase, the rubber is excellent in abrasion resistance without reducing low hysteresis loss property. Further, the rubber is low as well in compound Mooney viscosity and is excellent in processability (as shown in Examples 3 and 4, lines 7-11 at page 20 of the specification).

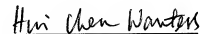
In Comparative Example 1 at page 17 of the specification, no enzyme treatment (namely, no decomposition of glucans) of the latex is conducted. Since the references do not teach or suggest the decomposition of glucans, Comparative Example 1 is representative of the references. Therefore, the Examples and Comparative Example of the specification demonstrate unexpectedly superior results provided by the present invention.

In view of the above, reconsideration and withdrawal of the rejections based on Kawamura et al or Galimberti et al or Ichikawa et al are respectfully requested.

Allowance is respectfully requested. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,



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CUSTOMER NUMBER

Date: June 16, 2009

## Proleather FG-F

*Proleather FG-F is a new proteolytic enzyme preparation developed by Amano Enzyme Inc..*

*This enzyme is manufactured by unique fermentation process of a selected strain belonging to *Bacillus subtilis*.*

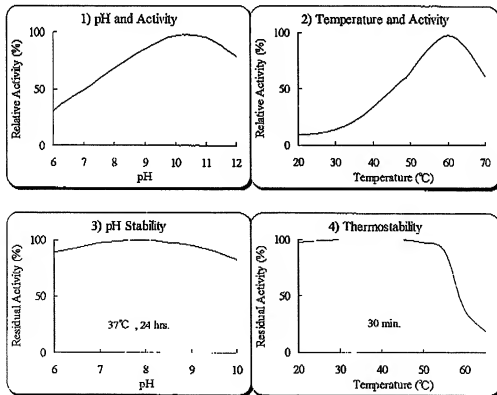
### CHARACTERISTICS

- 1) Proleather FG-F is an alkaline protease, and especially effective at pH 9.0-10.0.
- 2) Proleather FG-F is very stable in wide range of pH (6.0 - 10.0).
- 3) Proleather FG-F is very stable to heat.
- 4) Proleather FG-F is free-flowing fine granule for easy and safe handling.
- 5) Proleather FG-F has an excellent solubility in water.

### STANDARD

Proleather FG-F (protease activity): not less than 10,000 u/g (pH 10.0) by Amano's test method.

### PROPERTIES



The information and recommendations contained herein are to the best of our knowledge reliable according to the current scientific and technical level. However, depending upon use method and/or conditions, nothing herein is to be construed as a warranty or representation in respect of otherwise, including freedom from patent infringement. Users shall make their own test and investigations for their particular purposes. We do not accept any liability for any loss, damage or infringement arising from the use of information and recommendations contained herein.

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Attached Paper 2

AMANO PRODUCTS-03

DIGESTIVE  
ENZYME

**CELLULASE AP**

**Amano Enzyme Inc.**

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**CELLULASE AP** (Celluletic enzyme preparation)

"CELLULASE AP" is a digestive enzyme preparation, which contains cellulolytic enzymes.

"CELLULASE AP" is manufactured by unique fermentation of a strain belonging to *Aspergillus niger*, extraction and purification and standardization with a suitable diluent.

Dextrin is usually used as the diluent. Lactose can be used as well.

Three principal nutrients (carbohydrates, proteins, lipids), vitamins and minerals are included in foods and drinks.

The nutrients in plant cells are contained inside cell wall, and cells are glued each other with intercellular space substances (cellulose, pectins etc).

There are no enzymes which digest plant cell walls and intercellular space substances in human's digestive juice.

The nutrients in plant cells, therefore, can not be utilized sufficiently if people do not masticate foods well or suffer from gastrointestinal troubles.

"CELLULASE AP" has optimum pH around 4.5, and is stable at pH 2.5-8.0 so that its acts satisfactorily in both stomach and the intestines.

"CELLULASE AP" has 2 varieties which are different in specifications as follows.

**CELLULASE AP3**



## Standards of "CELLULASE AP"

Standards	CELLULASE AP3
Description	It occurs as a light yellow to light brown powder.
	It has a slight peculiar odor and taste.
	It is soluble in water, insoluble in ethanol.
Purity Test	
(Heavy metal)	Not more than 50 ppm
(Arsenicum)	Not more than 2 ppm
Loss on drying (1g, 105°C, 4hours)	Not more than 10.0%
Residue on ignition	Not more than 15.0% (1g)
Digestive power	
Cellulose saccharifying activity (pH 4.5)	1200 ~ 1800 u/g

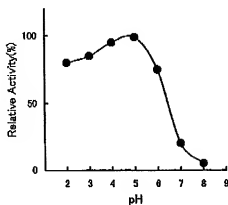
Caracters of "CELLULASE AP"
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## ① pH and Activity

(Procedure)

Substrate solution is prepared with each buffer solution\*.

Relative activity is shown as 100% at pH 4.5.



## ② pH and Stability

(Procedure)

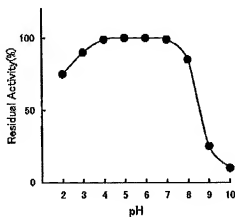
Add 1ml of 1.0% "CELLULASE AP3" solution to 9ml of each buffer solution\* and incubate at 37°C for 30 minutes.

Then, add 0.1N hydrochloric acid or 0.1N sodium hydroxide TS to a just to pH 4.5, and determine the enzyme activity.

Residual activity(%)=  $b/a \times 100$ 

a : enzyme activity before incubation

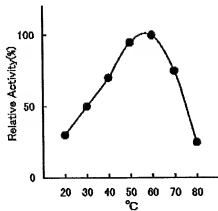
b : enzyme activity after incubation



- \* pH 2.0-3.0 : 1N hydrochloric acid-sodium acetate buffer solution  
 pH 4.0-6.0 : 1N acetic acid-sodium acetate buffer solution

## ③ Temperature and Activity

Relative activity is shown as 100% at 55°C .

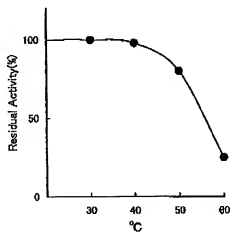
④ Temperature and Stability (on enzyme solution)  
(procedure)

Incubate 1.0% "CELLULASE AP3" solution at each temperature for 30 minutes, then determine the enzyme activity.

Residual activity(%)= $b/a \times 100$

a : enzyme activity before incubation

b : enzyme activity after incubation



## ⑤ Temperature and Stability (on enzyme powder)

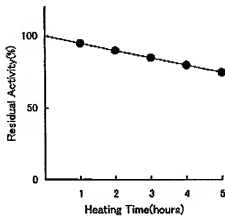
## (Procedure)

Heat powder of "CELLULASE AP3" in opening condition at 105°C, cool to room temperature, then determine the enzyme activity.

Residual activity(%)= $b/a \times 100$

a : enzyme activity before heating

b : enzyme activity after heating



Sequential stabilities of "CELLULASE AP"
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## ① Influence of heat

(Procedure )

Put the sample in an airtight glass container, keep at 5°C, room temperature and 40°C, and determine the enzyme activity after the storage of months mentioned below.

Residual activity(%)=  $b/a \times 100$ 

a: initial enzyme activity

b: enzyme activity after storage

R T : Room temperature

	month	1	3	6	9	12	18	24	30	36
Cellulose Saccharifying Activity (%)	5 °C	100	97	95	93	91	90	88	87	85
	R T	100	95	92	91	89	85	82	80	77
	40 °C	98	90	85	80	78	73	70	66	60

## ② Influence of light

( Procedure )

Influence of scattered light

Put the sample in an airtight glass container, keep at the window abiding the straight sun light.

Influence of fluorescence light

Put the sample in an airtight flat glass container, keep at the distance of 25cm under the fluorescence light (10W×2).

Determine the enzyme activities after the storage of months mentioned below.

Residual activity(%)=  $b/a \times 100$ 

a: initial enzyme activity

b: enzyme activity after storage

S L : Scattered light F L : Fluorescence light

	month	1	2	3	6	9	12
Cellulose Saccharifying Activity (%)	S L	100	98	95	93	90	88
	F L	100	97	95	92	90	87

## ⑧ Influence of humidity

( Procedure )

Put the sample in an opening glass container, keep in thermo-hygrostat under the following conditions and determine the enzyme activity after the storage of months mentioned below.

Residual activity (%) =  $b/a \times 100$

a: initial enzyme activity

b: enzyme activity after storage

month			1	2	3
Cellulose Saccharifying Activity (%)	25°C	58 RH %	100	100	97
	25°C	75 RH %	98	95	93
	25°C	93 RH %	92	80	77
	40°C	53 RH %	97	90	86
	40°C	75 RH %	72	58	45

The information and recommendations contained herein are to the best of our knowledge reliable according to the current scientific and technical level. However, depending upon use needed under condition, nothing herein is to be construed as a warranty or representation in respect otherwise, including freedom from patent infringement. Users shall make their own test and investigation for their particular purpose. We do not accept any liability for any loss, damage or infringement arising from the use of information and recommendations contained herein.

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